On the Biology and Structure of the Larvae of Hydrophilus caraboides L.

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With Plate 27 and 16 Text-figures.

In May 1918 I captured in the vicinity of Petrograd some cocoons of a hydrophilus beetle, one of which I kept for breeding purposes. On June 13 there emerged about fifty small larvae very similar to Hydrophilus caraboides. These latter are characterized by the presence of a pair of lateral (pleural) appendages covered with a dense brush of hairs on each of the seven abdominal segments (Text-fig. 13, *pla*).

In my larvae (Text-fig. 1) these pleural hairy appendages were also present, but with the difference that each appendage bore on its summit a long thin hair. This peculiarity caused me to look for other differences between my larvae and the description of the larvae of Hydrophilus caraboides, as given by Schiödte in his paper, 'De metamorphosi eleutheratorum observationes; bidrag til insekternes undviklingshistorie', 1861.

These differences may best be pointed out by a parallel comparison of the text of Schiödte's diagnosis and the description of the newly emerged larvae as observed by me, as follows:

Hydrophilus caraboides Schiödte Caput obovatum.	Hydrophilus caraboides —a larva at first stage. Head irregularly tetragonal, shaped rather like a trapezium turned with its base forward, and with broken sides.

Antennae articulo primo longissimo, tenui, ciliato (Text-figs. 6, 7, *b*), secundo et tertio tenuibus, pusillis, subaequalibus.

First joint of antennae long, slender and flat, inner margin with seven very distinct teeth (Text-fig. 5, 7, *a*), giving the joint a saw-like aspect. Second and third joints almost of equal length, each being about three times shorter than the first. Second in the middle of its outer margin with an elevation bearing a sense-organ in the shape of a chitinous ringlet with hairs in the centre.

Antennae scapo longissimo ciliato, natatoriae.

Mandibulae elongatae, falcatae, acutissimae, subaequales, dente medio valido, duplici, acumine, posteriore acuto, priore lanceolato, maiore (Text-fig. 6, *md*).

Antennae scapo longissimo, serrato, masticatoriae.

Mandibles shaped as a sharp, bent, and strong sickle with secondary teeth on its concave surface, the first largest, lancet like; the middle tooth tetragonal with a concave free surface and sharp prominent edges; hind tooth in the shape of a small tubercle at the base of the middle tooth.

Stipites maxillares gracillimi, longissimi, palpos labiales excedentes. Palpi maxillares graciles: stipes gracilis, recurvus, articulo primo triplo longior.

Mentum (Text-fig. 4, *m*) amplum, fornicatum, e basi sensim, decrescens latitudine, angulis dentiformibus, lateribus serrulatis. Stipes palporum labialium elongato-quadratus, basin versus angustior. Palpi labiales (*pll*) articulo priore brevissimo. Ligula elongata, acuminata (*lg*).

Mentum (Text-fig. 3, *m*) bell-shaped, both its fore angles protruding in the form of sharp teeth. Side margins smooth, slightly S-shaped. Stipes compared with the mentum much more developed than in the mature larva of H. caraboides, and in the form of an elongate rectangle which in its fore part is wider than behind. The base of the ligula occupies almost half of the width of the anterior margin of the mentum (in the mature larva of H. caraboides only one-fourth). Tip of the ligula is somewhat bilobate (*lg*).

Scuta prothoracica concreta, completa, integra. Scuta mesothoracica discreta, tergum medium occupantia, triangula, apicem truncatum versus constructo-attenuata. Scuta metathoracica discreta, incompleta, apice incurva.

The prothoracic tergite has the shape of a continuous shield of an almost regular quadrangle, the posterior corners being obliquely cut or rounded (the tergite of the mature larva of H. caraboides is conspicuously narrowed in front and in outline much like a truncated cone). The meso- and metathoraces bear each on their tergites two triangular shields, the apex of which is not bent.

Praeterga abdominis integra. Terga obscurius plicata, scutis verruciformibus, cylindricis. Appendices pleurarum abdominis praeter octavum par elongatae, inaequales, ciliis gracillimis vestitae, natatoriae; appendices octavi paris deorsum flexae, acuminatae, sinuosae, suspensoriae.

Abdomen densely hairy (Text-fig. 1). Tergites with four warty tubercles which are also covered with hair, and with a long seta on the summit. Before the middle pair of tubercles there is a pair of brown shields of strong chitin, also bearing long setae.

Pleural appendages in shape of cylindrical prominences, and densely covered with hair, resembling a lamp-brush. From the summit of each appendage there emerges a long and thin seta (Text-fig. 1).

Above the base of each lateral pleural appendage there is a small warty tubercle with a long seta.

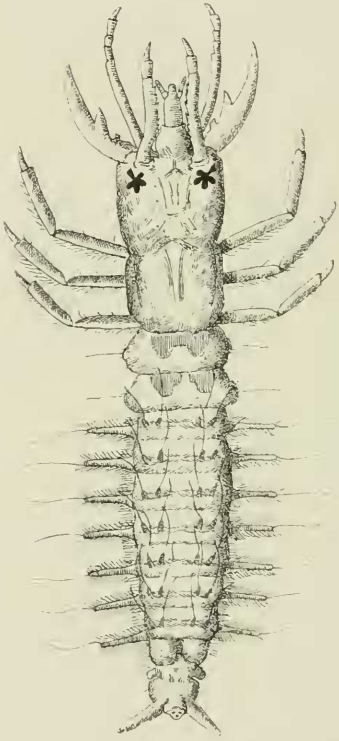
The examination of more advanced larvae of Hydrophilus caraboides, i.e. such that had already moulted, convinced me of the accuracy of Schiödte's description. The antennae of such larvae are in fact hairy on their inner edges, and the base of the lamella of the under-lip has a dentate edge. The long setae on the tips of the pleural appendages are partially present but not conspicuous.

On the whole one might say that from the egg of Hydrophilus caraboides emerges a larva which in its successive

ecdyses changes some structural characters, especially in the antennae, the under-lip, and partly the pleural appendages.

In the literature the excellent drawing of the mature larva given by Schiödte is generally adopted ; but I have not been

Text-fig. 1.



Hydrophilus caraboides. A larva, first stage.

able to find any figure of a young larva, therefore I here adduce its figure, drawn unfortunately from a specimen preserved in spirit, on account of which the abdomen seems shorter than it is in reality (Text-fig. 1). It is true that it easily extends, a fact which causes some variation in its length even under normal conditions.

These facts found by me caused me to investigate the larvae

of Hydrophilus caraboides more thoroughly. The larvae were brought alive into Duboscq's mixture (15 c.c. of 1 per cent. solution of picric acid in 90 per cent. alcohol +6 c.c. of formalin and 1·5 c.c. of ac. aceticum glaciale), and after they had ceased to move they were cut in two with scissors.

The fixation lasted from twelve to sixteen hours. After this the larvae were brought through several portions of 85 per cent. alcohol to wash out the picric acid. Further, they were embedded in paraffin. The sections were stained with iron haematoxylin, Giemsa stain, and Unna's polychrome methylene-blue.

On the Structure of the Digestive Apparatus.

The larvae of H. caraboides are distinguished from all other aquatic insects by their unusual way of taking food. Observations on this point have already been made by several naturalists, and Brocher (1913) even figures (semi-diagrammatically) a larva of H. caraboides taking food (fig. 68). According to a more detailed description of Portier (1911) this process takes place as follows.

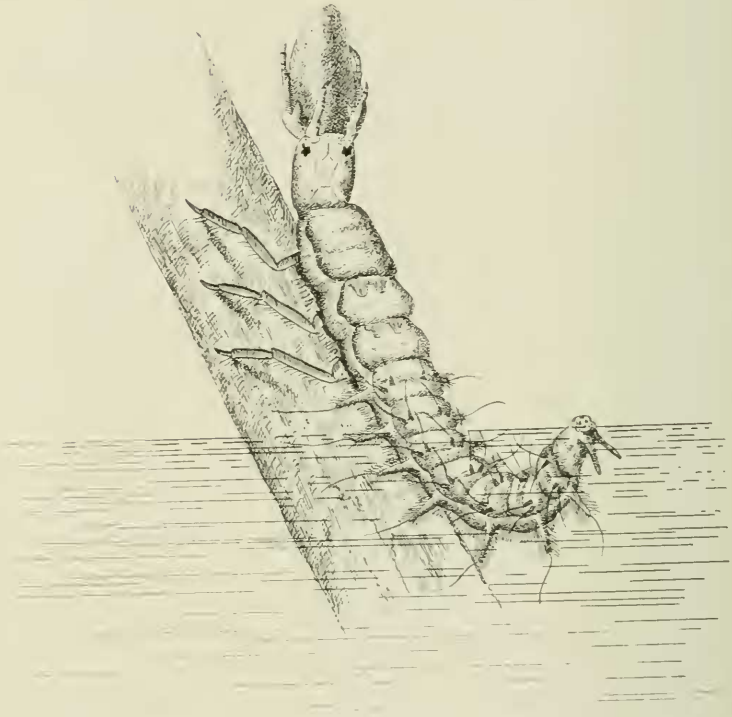
Having caught with its powerful and sharp mandibles its prey (for instance a small crustacean, an insect, &c.), the larva of Hydrophilus 'se dirige vers une plante aquatique ou contre la paroi du vase qui la renferme, si aucun objet ne flotte à la surface de l'eau. Au moyen de ses pattes antérieures, elle s'accroche à une aspérité quelconque située au-dessus de la surface, puis elle renverse sa tête en arrière sur son dos. On comprend que, dans cette situation, sa tête émerge complètement. C'est, en effet, le résultat auquel la larve semble tendre. . . . La proie ayant été ainsi élevée au-dessus de la surface, on voit l'appareil masticateur entrer en jeu, la chitine est perforée par les tubercules chitineux qui garnissent les mandibules et le sang rouge de la larve de Chironomus[1] se met à couler.

'A ce moment, un flot de liquide noir envahit les organes

[1] Portier describes the feeding of a larva of H. caraboides on a larva Chironomus.

buccaux ; c'est le liquide digestif qui a été injecté à travers l'œsophage par l'intestin moyen. Ce liquide noir adhère par capillarité à la proie ; il est contenu dans une espèce de corbeille formée par les différentes pièces de l'armature buccale

Text-fig. 2.



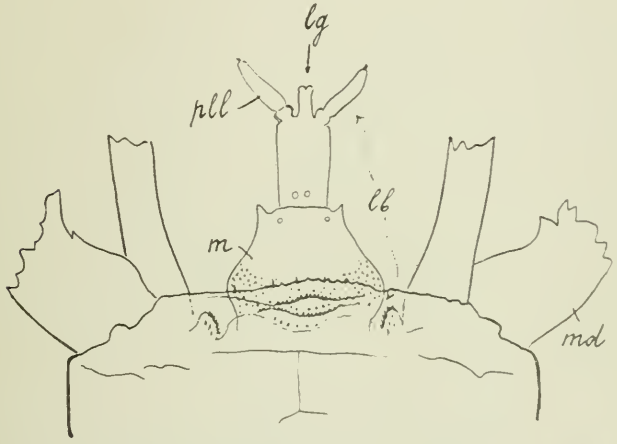
Larva of Hydrophilus caraboides devouring a cypris.

si développée chez ces larves. La proie est ainsi complètement baignée dans la liqueur digestive. Après quelques instants de contact, le liquide chargé maintenant des produits de la digestion est réabsorbé ; il passe par l'œsophage, puis dans l'intestin moyen dont il gagne immédiatement la partie postérieure, ainsi qu'on peut s'en assurer sur les jeunes larves, ou sur celles qui viennent de changer de peau, toutes deux possé-

dant des téguments d'une transparence parfaite' (Portier, 1911, pp. 175–6).

The inconvenience of the attitude during the feeding process (Text-fig. 2) is increased for the larva of H. caraboides by the necessity of breathing. For this purpose the larva bends its body in such a way that the posterior end of the abdomen

Text-fig. 3.



Anterior margin of head and lower lip of newly-hatched larva. Base of lower lip (*lb*) with simple lateral margins (*m*). On the sides spined folds of the connective chitinous cuticula (*t*). *md*, mandible; *lg*, ligula; *pll*, palpi labiales. Zeiss, ob. AA, oc. 0.

bearing the breathing aperture touches the surface of the water (Brocher).

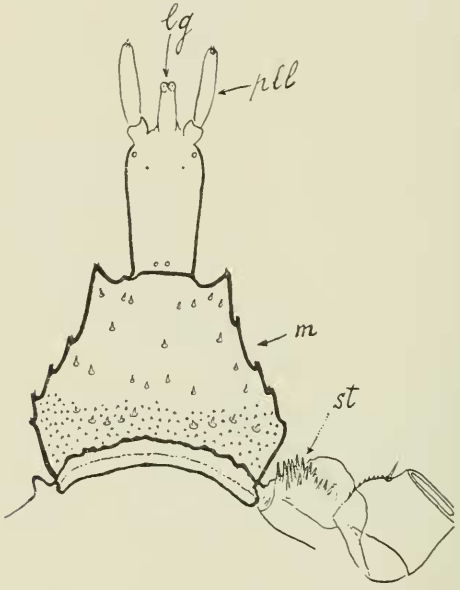
In connexion with these peculiarities we shall look into some of the details in the structure of the digestive apparatus of the larva of H. caraboides.

The well-developed and powerful mouth-parts of the larva serve to seize the prey and to treat it mechanically. Having seized some suitable animal, the larva of H. caraboides pierces it with the teeth of its sickle-shaped mandibles (Text-fig. 6, *md*), tearing open the integument of its prey. After this the larva assumes the habitual feeding attitude and begins to work energetically with all its mouth-parts, closing and

reopening them. Gradually the prey crumples and is thrust in deeper and closer to the oral opening.

In the grinding and kneading not only the mandibles take part but also the antennae, which in the larva at the first stage have a special contrivance for this purpose in the form of a row

Text-fig. 4.



Lower lip of mature larva. Dentation of lateral margins and sensory cones of upper surface of base of lip (*m*). On the sides spined folds of the connective chitinous cuticula (*st*). *lg*, ligula; *pll*, palpi labiales. Zeiss, ob. AA, oc. 0.

of sharp teeth on the inner edge (Text-figs. 5, 7, *a*). These teeth are chaetoids (after the terminology of Nasonov, 1901), i. e. they are evaginations of the integument of the larva and the body-cavity is continued into these appendages. The antennae of the mature larva do not take any energetic part in the mastication of the prey, having on the inner edge about three rows of long flexible setae (Text-figs. 6, *a*, 7, *b*), which are therefore inconvenient for tearing or kneading the prey.

The under-lip (Text-figs. 3, 4, *lb*) serves as a plate on which the preparation of the food is effected. During the feeding process the labial palpi (Text-figs. 3, 4, *pll*) are all the time in motion. Owing to the work of the mouth-parts the body of the captured animal is kneaded, with the result that the liquid

TEXT-FIGS. 5, 6.

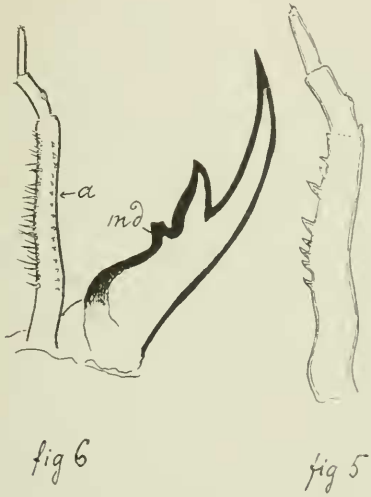


Fig. 5.—Right antenna of newly-hatched larva. The inner edge is serrated ; outer edge simple. Winkler, ob. 1, oc. 2.

Fig. 6.—Antenna and mandible of mature larva. Inner edge of antenna set with hairs, outer edge bears a row of short spines (*a*). *md*, mandible. Winkler, ob. 1, oc. 0.

parts or those being in a state of emulsion can be sucked up by the larva.

But into the alimentary system of the larva are introduced also the solid constituent parts of the prey after they have been treated with the ferments of a black digestive fluid which is exgurgitated from the intestine.

The whole of the feeding of the larva of H. caraboides takes place outside the water, a fact that, as has already been said, depends on the structure of its mandibles, which, in contrast to those of the larvae of the Dytiscidae, are not pierced

by a canal (Text-fig. 8, *md*) serving for the pumping of the sucked-up liquid into the intestine.

The prey is in the air while the larva of H. caraboides is feeding, and in order that the liquid parts may be sucked up it is necessary that the prey should be tightly pressed to the mouth-aperture of the larva.

The oral slit is situated between the upper- and under-lips of the larva (Text-fig. 9, *ap*), and the hind third or quarter of the mentum is covered with minute chitinous spines (Text-figs. 3, 4, *m*). Larger spines are found sticking out as a bundle on the tuberculiform elevation on the connective chitinous (Text-fig. 4, *st*) covering at the base of the lower jaws.

Thanks to these peculiarities, the deeper parts of the periphery of the oral aperture adhere tightly to the adjacent parts of the seized prey, and when the mouth-parts are closed the slits between their bases are filled up by soft connective chitin. Thus is obtained a hermetic contact of the oral opening with the body, the liquid parts of which have to be sucked up.

The oral armature of the larva of H. caraboides does not play a mechanical part only.

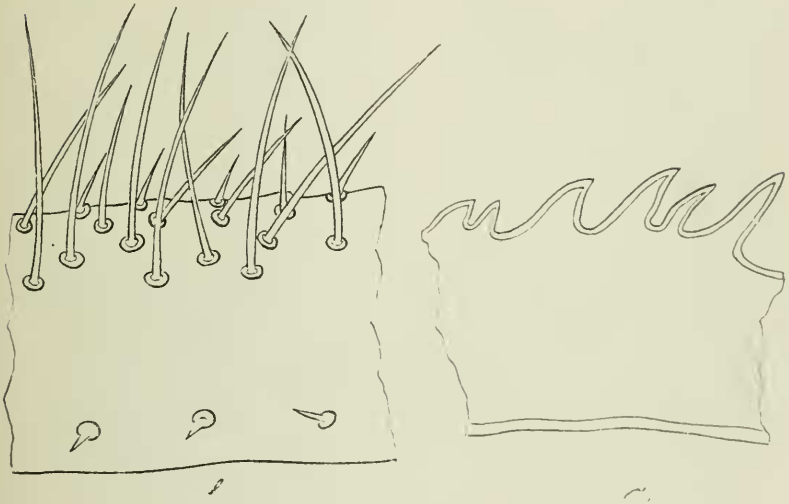
Very likely it serves also for receiving gustatory irritations, as on the lower lip there are scattered special sensory organs in shape of large pits in the chitinous cuticula. Ring-shaped sense-organs are found also on the outer edge of the third antennal joint ; on the tips of the antennae and labial palpi there are situated bundles of special chitinous spines (sensory organs ?) ; it may be remembered that on the upper surface of the mentum of the mature larva there are symmetrically scattered short and stout spines, bordered at their base ; analogous formations are found also on the dentate lateral prominences of the mentum (Text-fig. 4, *m*).

The prey, prepared mechanically (by the mouth-parts) and chemically (by the ferments of the digestive fluid), enters in parts into the feeding larva. The work of the mouth-armature one is inclined to compare superficially with chewing. This comparison obtrudes itself upon one's mind when one sees how energetically the larva kneads the prey with its mandibles,

turns it round and round and squeezes it. The liquid parts of the food are sucked up at this time by means of a special sucking apparatus of which we give a description, based on the study of series of transverse and longitudinal sections of the head and thorax of the larva of H. caraboides.

The fore-gut of the larva described runs as a straight tube from the oral aperture to its point of mergence into the stomach,

Text-fig. 7.



Parts of antennae of a newly-hatched (*a*) and a mature (*b*) larva at the same magnification. Zeiss, ob. DD, oc. 0.

which takes place in the thorax. In this fore-gut three divisions can be recognized, viz. the fore part reaching to the nervous ganglia; the middle part corresponding with the pharyngeal ring of the nervous system; and the hind or post-cerebral part, or the oesophagus proper.

The fore part is a dorso-ventrally flattened tube (Text-fig. 10) with sharp lateral edges. Its upper surface is covered with transverse muscular bundles (s_1), which are fastened to the sharp edges of the intestine. These muscles can, as I believe, be regarded as sphincters of a suctorial apparatus, for when

contracted they press the upper wall of the tube to the convex under-surface of the same. The antagonists of these muscles are the muscular bundles, extending from the upper surface

TEXT-FIGS. 8–11.

From a series of transverse sections of the head of a larva of the first stage. Zeiss, ob. AA, oc. 0.

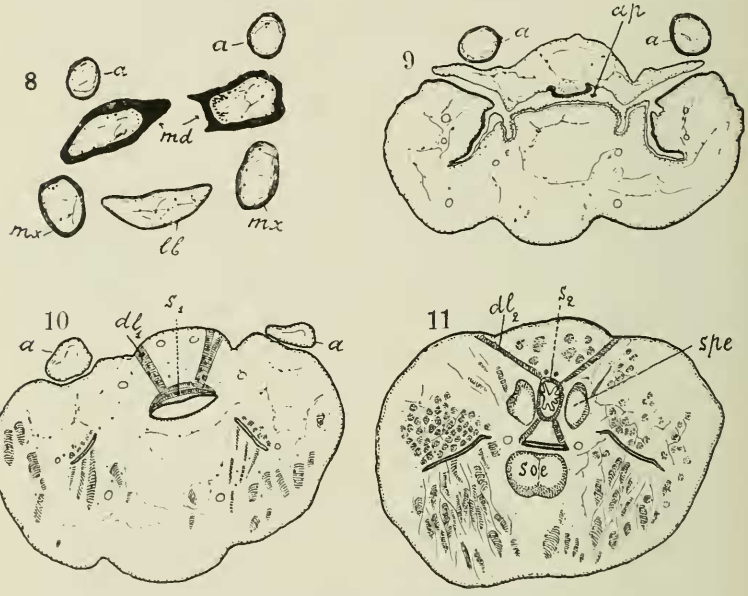


Fig. 8.—Transverse section through mouth-parts. Mandibles (*md*) without inner canals ; *mx*, maxilla ; *a*, antenna ; *lb*, labium.

Fig. 9.—Transverse section at level of union of mandibles with head. The oral aperture (*ap*) with its spiny inner surface is visible.

Fig. 10.—Section near the region of antennae (*a*). Suctorial apparatus—sphincters (s_1) and dilatators (dl_1), attending fore part of intestine.

Fig. 11.—Section at level of cephalic nervous ganglia (*soe*, *spe*). Fore-gut with dilatators (dl_2) and sphincters (s_2) of pharynx.

of the intestine to the upper wall of the head (Text-fig. 10, dl_1). When contracted they lift the upper wall of the tube, and, therefore, increase its cavity ; these levators might be called suctorial muscles. There are several pairs of them.

Farther backwards, the alimentary tube when compressed takes the shape of a groove, the upper edges of which are tied together by muscular bundles; the levators running in an oblique direction are located at the bottom of the groove, and between them, just in the middle, there are muscular bundles which, perhaps, serve for the contraction of the part of the intestine here described.

Nearer to the level of the brain-ganglia (Text-fig. 11, *spc*, *soc*) the structure of the intestine is different. The walls of the tube grow thinner. In section it looks like a strongly plicated ring surrounded by circular muscular filaments (second sphincter, Text-figs. 11, 12, s_2). They alternate with bundles of the radiating dilatators of the middle part of the fore-gut. There are four groups of dilatators—two upper (dl_2) and two lower ones; the upper groups are fastened to the upper wall of the head, the under ones go round the sub-oesophageal ganglion and, as it appears, partly terminate on the chitinous fold of the endoskeleton which lies above the fore end of the sub-oesophageal nervous ganglion. Behind the pharyngeal ring the intestine retains its form of a thin-walled tube strongly plicated longitudinally, clad in the usual muscular covering. The fore-gut opens directly into the mid-gut and does not form any valve or cardiac fold at the border of the stomach.

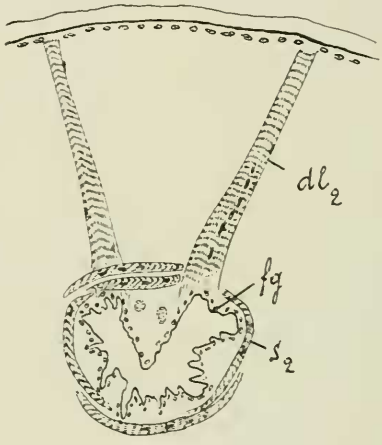
On the whole it may be said that the fore-gut of the larva of H. caraboides is provided with two muscular apparatuses: (1) the suctorial apparatus, which sets into action the first group of sphincters and levators (Text-fig. 10), and (2) the pharyngeal apparatus (the second suctorial apparatus), the sphincter and dilatator of which act on to the middle part of the fore-gut (Text-fig. 11).

Having acquainted ourselves with the structure of the fore-gut of the larva of H. caraboides we can picture its functions as follows.

With the contraction of the second group of sphincters (Text-fig. 11, s_2) the middle portion of the fore-gut is tightly closed. Then the levators of the suctorial apparatus (Text-fig. 10, dl_1) contract and lift up the upper wall of the digestive

tube, at the same time there develops in the suctorial apparatus a negative pressure, which causes the liquid matter of the prey, which is squeezed out by the mandibles, to enter the apparatus and fill its cavity (the sucking act). The next moment of feeding is characterized by the relaxation of the second group of sphincters (Text-fig. 11. s_2) and the contraction of the dilatators (Text-fig. 11. dl_2) of the pharyngeal apparatus

TEXT-FIG. 12.



Dilatators (dl_2) and sphincters (s_2) of pharynx (fg). Zeiss, DD, oc. 0.

(of the middle portion of the fore-gut): at this moment the corresponding part of the intestine is strongly dilated. Simultaneously the levators of the suctorial apparatus (Text-fig. 10. dl_1) relax and its sphincters contract (s_1). As a result the apparatus is closed, and the food which it contained is pressed into the dilated oesophagus (the act of forcing).

Then sets in the third act of food-taking—the closing of the pharyngeal apparatus (the suctorial apparatus being closed): the relaxing walls of the intestine force the food into the oesophagus and the stomach. In this way the larva makes a gulp of food (the act of swallowing). The whole act of food-taking

consists of the successive action of the suctorial and pharyngeal apparatus in the order described above.

Generally speaking, one may say that in the head of H. caraboides the fore-gut is organized as a forcing pump; during the suction the part of sucker of the pump is played by the upper wall of the suctorial apparatus, which is lifted up by the levators: simultaneously the pharyngeal apparatus contracts its sphincters and acts as the valve of the pump, preventing the food already swallowed into the stomach from being sucked back again.

At the next act—the forcing—the action of the different parts of the apparatus change. The pharyngeal apparatus acts as a sucking contrivance, and the proper suctorial apparatus comes to a state of rest and, pressing on the liquid it contains, produces a forcing action. This liquid cannot re-enter the body of the prey, as the latter, being worked into a lump, is tightly pressed towards the oral aperture and is, moreover, firmly squeezed with the mandibles.

Consequently not only liquid parts of the prey are swallowed, but also solid portions of its body, as for instance bits of tracheae, bits of the chitinous cuticula, &c. I have found similar remnants in the rectal sac of the larva of H. caraboides (Pl. 27, fig. 1, *ch*), a fact which would be impossible in the case of typical suctorial insects, as for instance the larva of Dytiscus.

In the latter the food enters the suctorial apparatus by canals in the mandibles which remain perfectly immovable during the suction of food; as the latter is submitted to a chemical treatment by ferments only, a mechanical action on the food-stuff is absolutely excluded.

It is interesting that the general idea of a sucking and forcing apparatus (consisting of two contrivances—the suctorial apparatus proper and the pharyngeal apparatus) is repeated with insignificant modifications in different arthropods, for instance in scorpions (Pavlovsky, 1917; Pavlovsky and Zarin, 1918), in Arachnida (Schimkevitsch, 1884), and insects (lice (Pavlovsky, 1906; Sikora, 1916), bugs (Voronkov, 1907),

aphids, gnats (Nuttall and Shipley, 1903), and many others). This likeness may be explained by a convergence, caused by such a typical action, from the physical point of view, as the suction of liquids.

The gastric fluid (black in colour), which is poured over the seized prey, is regurgitated from the stomach under the influence of an antiperistaltic movement of its walls. The regurgitation is favoured by the absence of any adjacent cardiac valves.

The mid-gut or stomach has, as has been pointed out by Portier, many short blind papillae, or cryptae, formed by the evagination of the epithelial wall of the intestine into the body-cavity. On my preparations closely set mitoses of the epithelium were also visible.

It is instructive to compare the size of the cells of the intestine in just emerged and mature larvae of H. caraboides. A better representation than any description can be given by figs. 3 and 4 (Pl. 27), made at the same magnification. On the longitudinal section of the crypt (Pl. 27, figs. 3, 4, *crp*) of a young larva the number of cells is counted by units, and in the mature larva by tens. The cells themselves are much larger in the latter; the association of these causes has an influence on the size of the cryptae. The nuclei of the cells of the cryptae and the intestine itself in larvae of different age differ in size from each other comparatively less than the size of the cells themselves. The latter fact depends upon the real growth of the cells as well as their secretive action.

The growth of the cells in dependence on the growth of the larva is best demonstrated in the muscular fibres of the cover of the intestine. In the larva of the first stage the muscular fibres are very thin (Pl. 27, fig. 3, *mt*); whereas in the mature larvae they are fifteen to twenty times stouter (Pl. 27, fig. 4, *mt*). This growth is explained by the increase of the myofibrillae, which are differentiated in the sarcoplasm of the muscular cells.

The lack of material did not allow me to study the question of the regeneration of the intestinal epithelium, which in the adult beetle is periodically cast off and replaced by a new one,

which develops by karyokinesis of the cells of the cryptae (Rengel, 1898).

Even in the young larva the intestine for all its shortness forms a loop in the hind part behind the entering point of the Malpighian tubules. In the mature larva the latter are characterized by the accumulation of a large number of pigment grains (Pl. 27, fig. 5, *pg*) which concentrate in the basal half of the cells, and surround their nuclei. The parts of the cells which are turned to the lumen of the tubule look in dissection like a broad pale border with a slight indication of a faint striation (the Stäbchensaum) without accumulations of pigment.

It is of interest to compare here the size of the cells of the Malpighian tubules in the young and mature larvae of H. caraboides, drawn under the same conditions (Pl. 27, figs. 5, 6). In the young larva the nuclei occupy nearly the whole of the cells, which do not yet contain accumulations of pigments.

The ileum enlarges nearer to the end of the body into a rectal sac the structure of the walls of the latter being not all alike. The anterior wall of the sac, which borders on the ileum, has the same structure as the latter. It consists of a cylindrical, rather high epithelium, the free surface of which shows a conspicuous striation (Pl. 27, fig. 1, *ep*) ; such a structure of the protoplasm is not an exceptional one ; analogous relations are found for instance in the ileum of the bee (E. Pavlovsky and E. Zarin), the rounded nuclei with minute thickly-crowded grains of chromatin occupying the middle part of the cells or in some places moving nearer to the surface of the epithelium.

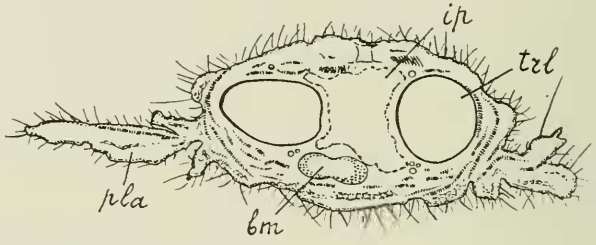
The latter forms on the back of the rectal bladder a thickened disc, the edges of which are strictly defined, as the cylindrical epithelium abruptly passes over into flat epithelium (Pl. 27, fig. 1, *epp*), which is of usual occurrence in the rectal part of the intestine of many insects.

In the young larva the cylindrical epithelium of the rectal sac (as well as the ileum) is correspondingly lower than in the adult larva (Pl. 27, fig. 2, *ep*), a fact which depends chiefly on the degree of growth of the cells, as no secretory processes, which could

influence the size of the cells, have been observed in the rectal sac of either the young or the mature larvae.

The greater part of the hind-gut is occupied by the rectal sac, which is located between the main tracheal side-trunks (Text-fig. 13, *trl*). The high epithelium of the rectal sac has not the significance of rectal glands, which are present also in the larvae of some insects with heterometabolous development in the shape of longitudinal stripes of high cylindrical epithelium (for instance, the rectal glands of Orthoptera).

Text-fig. 13.



Transverse section through one of hind segments of abdomen with pleural appendages (*pla*). Rectal sac (*ip*) between main tracheal trunks (*trl*). *bm*, brain. Zeiss, ob. AA, oc. 0.

The presence of a cylindrical epithelium in the rectal sac of H. caraboides is simply explained by the fact that in this instance the anatomical border between the parts of the hind-gut, which can be defined by superficial inspection, does not correspond to the histological border between the tissues composing it.

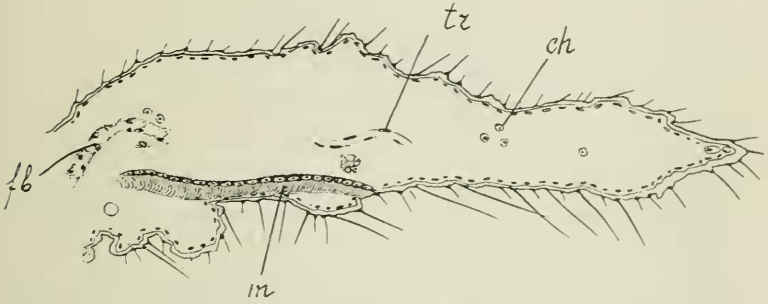
Analogous relations have been observed by me in the poison glands of Hymenoptera; its reservoir is similarly well differentiated, and histologically it is built of a wall which on one side bears a decidedly glandular character, and on the other of that flat epithelium peculiar to the duct of the acid gland (Pavlovsky, 1912).

Respiratory Organs.

Two main longitudinal tracheal trunks enter a common spiracle chamber (atrium stigmatique, Text-fig. 16, *at*) situated

in the hind end of the body, which opens into a single breathing aperture (*a*). The details of the structure and function of this part of the respiratory system are given in Portier's paper, who says of the larvae of Hydrophilus piceus, H. caraboides, and Hydrobius fuscipes, the following: 'Il n'y a point de faux stigmates apparents sur les parois latérales du corps, et pas non plus naturellement de ramifications trachéennes qui se rendent à cette région des

Text-fig. 14.



Longitudinal section of pleural appendage of abdomen. The tracheae (*tr*), muscles (*m*), fat-body (*fb*), and cells of haemolymph (*ch*) are visible. Zeiss, ob. DD, oc. 0.

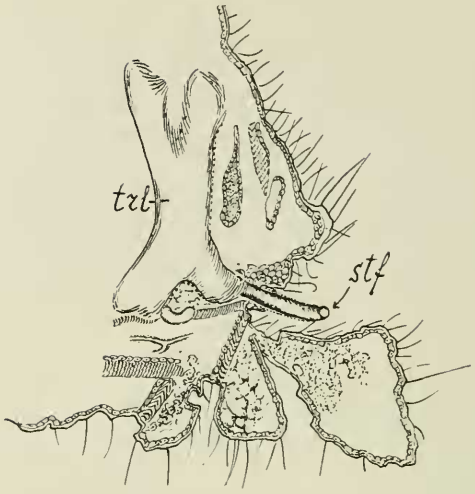
téguments comme on en voit chez les larves de Dytiscides' (loc. cit., p. 259).

This conclusion of Portier's is not precise, at least in the case of the larvae of H. caraboides, as the latter have nine pairs of lateral spiracles, two of which are on the thorax and seven on the abdomen.

The spiracles have an oval external outline, the latter showing two smaller ovals closely adjoining each other. The spiracles are located on the tops of conical evaginations of the integument, which in these places are covered with dark-brown chitin. These evaginations are situated on the sides of the body in the part of the pleural appendages of the larva (Text-fig. 15, *stf*). From the spiracles the initial narrow trunks of the tracheae start as a sort of vestibulum.

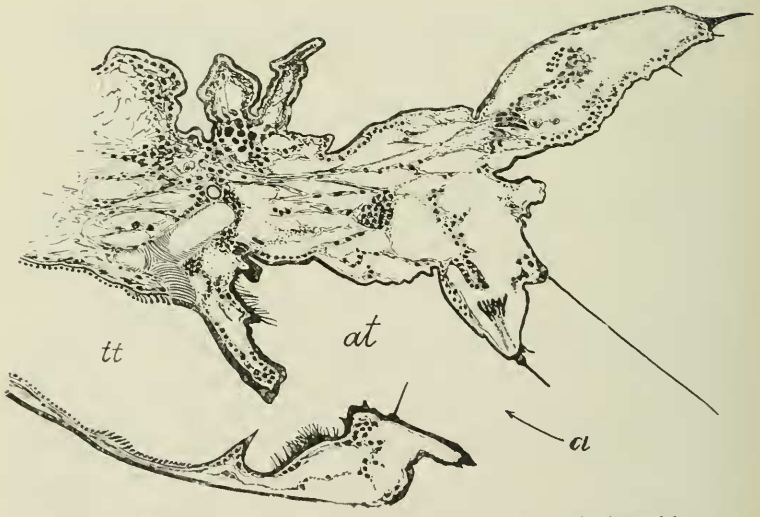
They are thick-walled and set inside with a dense brush of

TEXT-FIG. 15.



Transverse section of lateral wall of abdomen of larva of first stage through a closed spiracle (*stf*). *trl*. tracheae. Zeiss, ob. DD, oc. 4.

TEXT-FIG. 16.



Longitudinal section of hind end of abdomen with breathing apertures (*a*), atrium (*at*), and end of main longitudinal tracheal trunk (*tt*). Zeiss, ob. DD, oc. 0.

pillars, that is to say they have a structure similar in its idea to that of the spiracles and the beginning of the tracheae in other insects, as, for instance, in the caterpillars of Lepidoptera (Bombyx mori) (Verson and Guayat).

In the latter instance the inner brush-like structure of the vestibulum plays the part of a filter, serving to retain the dust from the air inhaled.

In the larva of H. caraboides the pillar-like cuticula of the initial parts of the tracheae evidently has no special application, since the spiracles themselves are closed by a chitinous membrane. That they are really closed, and therefore do not act as breathing apertures, can be well observed on a living larva immersed in a fixing fluid, as for instance Duboscq's mixture. The body is compressed by the action of the fixative, and the air comes forth from out of the spiracles in the form of silvery bubbles. Such bubbles appear in the larva of H. caraboides only on the hind end of the body, i. e. from the mouth of the tracheal chamber (atrium stigmatique). But the lateral spiracles join, as has been observed above, the main longitudinal tracheal trunks by means of narrow branches.

The tracheal trunks play also an accessory part—they serve as natatory air-bladders which facilitate the ascent of the larva and passive flotation near the surface of the water.

The larvae of H. caraboides are very sensible to lack of air. During the feeding process they must breathe; on account of this necessity the larva is obliged to assume a very peculiar and uncomfortable attitude when feeding, as is illustrated in Text-fig. 2.

If the larva is put into a small vial with water corked in such a manner that there is no bubble of air left inside the vial, and it cannot therefore find the surface of the water, it begins to make restless movements.

The larva starts up and meets with the glass wall of the vial. It struggles with the end of the abdomen, swims actively about, changes place in search of the surface of the water. Its restless movements become swifter, it turns along the axis

of the body, draws in the end of the body and swiftly pulls it out again, as if trying to tear off something closing its breathing apertures. Probably such movements are made by the larva also under natural conditions after moulting, in order to throw off parts of the old skin or the chitinous lining of the tracheae, which might be left sticking to the hind end of the body and thus diminish the breathing aperture or even altogether shut it.

After fifteen to twenty minutes the periods of movement of the larva alternate with periods of rest. The movements of the legs and the mouth-parts become convulsive, and are repeated more and more rarely. The larva rises passively and lies under the surface of the glass in a bent attitude with its belly up. In this case the significance of the tracheae as a natatory apparatus is manifested best. The immovable larva is not yet dead and can regain life if transferred into an open vial; the time during which a larva can be kept without fatal results without air varies and depends on various circumstances, as temperature, individual peculiarities of the larva, &c. In some of my experiments the larvae came to life again after having been deprived of air for two and even seven hours.

The question arises whether the pleural appendages (Text-fig. 14) of the body and the caudal appendages play an accessory part of tracheal gills, but I have not been able to clear this up for want of material and other reasons.

In conclusion of these remarks on the respiratory organs we might discuss the finer structure of the tracheal trunks of H. caraboides. As in all insects the chitinous lining (intima) of its tracheae bears filiform thickenings, the taenidia, the windings of which give the tracheae a transversely striated aspect. In the larva of H. caraboides the taenidia have not necessarily the aspect of a spiral thread, as in some places there are free ends of them between two adjacent windings; thus presenting a picture of structure similar to that observed by Minot (1879) in Hydrophilus (Hydrous) piceus (cited after Berlese, 1909).

The degree of independence, or rather of precision, of the

taenidia is disputed by R. Schneider (1902), who writes on the tracheae of Hydrophilus piceus as follows: '... eine faserartige Verdickung der Intima existiert aber nicht, es kann also nur von einer Spiralfalte geredet werden. Genaue Untersuchung zeigt folgendes. Zu unterscheiden sind die Furchen, welche eine Falte begleiten, ferner die steil aufsteigenden Faltenwände und die flache Faltendecke, deren Breite im Durchschnitt der einer Furche entspricht. An geschwärztem Materiale treten entweder die Wände als schwarze Striche, die parallel nebeneinander verlaufen, oder die Faltendecken als schwarze Streifen scharf hervor, während die Furchen immer mässig dunkel erscheinen' (loc. cit., p. 505).

When stained with iron haematoxylin, or Giemsa's stain, the taenidia of the main tracheal trunks of the larva of H. caraboides stand out vividly on the colourless ground of the chitinous cuticula, from which they are more separated, than the structures in the shape of grooves or folds.

In the taenidia just described I succeeded in observing character of their structure which bears evidence in favour of their strong individualization from the lining of the tracheae, of which the former are of course a product. Thanks to the comparatively considerable thickness of the taenidia, their heterogeneous structure is plainly visible in sections (transverse, or better in tangential sections). The taenidium is a thin-walled capillary tube, as the darker wall and the lighter lumen are easily distinguished.

This structure has a certain meaning. The walls of the tracheae must possess a considerable elasticity, as for breathing purposes their lumen must be open for the passage of air. Besides, they perform another function of a more mechanical significance, i. e. they are the ligaments that hold the organs in their mutual position. Finally, the tracheae represent elastic pillows, which lie between different internal organs.

The latter function of the breathing tubes is clearly visible in the larva of H. caraboides, since the greater half of the cross-section of the abdomen is occupied by the tracheae. Between the latter passes the hind intestine, which, when overfilled with excrements, presses on the adjacent walls of the

tracheae. These latter resist the pressure owing to the elasticity of the included air (which acts only when the breathing apertures are closed), as well as in consequence of the structure of the tracheal walls themselves. The taenidia having the structure of pipes resist any mechanical influence, in particular pressure, more completely than would taenidia of the same diameter if they were solid. An analogous principle in the resistance of materials is applied in the engineering practice. The forms of taenidia are not the same in different insects. Passing over the details we might point out that the taenidia of hemipterous Zaitha fluminea have the form of a groove, which structure gives the trachea a mechanical advantage.

On the Integument and its Appendages.

The body of the larva of H. caraboides is thickly set with hairs. These are of several kinds :

(1) Fine hairs ; the most numerous on the segments and the pleural appendages of the abdomen.

(2) The long terminal threads of the pleural appendages.

(3) The scarce setae.

(4) The setae on a pigmented and elevated base.

The latter are arranged in fours on the tergites of each abdominal segment, forming together four longitudinal rows.

In connexion with its dense hairiness the integument of the larva of H. caraboides possesses a high degree of sensibility. It is sufficient to touch one of the long hairs of the pleural appendages of the abdomen (Text-fig. 1) to make the larva instantaneously turn its head to the side where the irritation comes from and to seize the disturbing object with its mandibles.

These organs are useful in two respects : (1) They serve the larva for self-defence, as its whole body with the exception of the head and perhaps also of the thorax is very soft, easily vulnerable, and accessible to the attacks of different carnivorous inhabitants of fresh water ; receiving due notice of any possible danger, the larva gets time to put its defensive organs into action, i. e. its sharp and strong mandibles. (2) If a weaker or harmless creature happens by chance to touch one of the

long sensory hairs of the larva it becomes its prey, as it gets very little chance of escaping the murderous mandibles: in this latter case the sensory hairs attend indirectly the feeding wants of the larva.

The sensory hairs of the pleural appendages are of considerable length; in the young larva of the first stage the hair might be twice or thrice as long as the pleural appendage. In consequence the receiving surface of the body of the larva is considerably increased, and the latter is able to orientate itself better in the surrounding medium, both for taking measures of defence and for the capture of prey. The armature of the head of the larva is usually kept in readiness, i. e. all oral appendages are wide open and have only to contract at the suitable moment.

An analogous sensibility is found also in the hairs on the tergites of the body. When the irritation comes from above, the larva throws its head up and backwards with the same quickness and generally attains its end.

But the larvae of H. caraboides are not absolutely safe from peril. For instance, they are ready to devour each other if kept in close vials and fed unsatisfactorily. An examination of the skins of devoured larvae showed that they were all wounded in the tergites of the thorax only. This place is, so to say, the Achilles-tendon of our larvae.

The thoracic integuments are also not devoid of sensibility; but if the attacking animal succeeds in seizing it at once by the tergites of the thorax, the larva of H. caraboides finds itself in a defenceless position, because in this case it cannot throw back its head and put its mandibles into action.

In some of the larvae the pleural appendages and their terminal hairs were partly torn off. The aperture of the wounded places were shut by dark-coloured chitinous plugs of an evidently inflammatory origin. Doubtless the respective larvae had been in a position endangering their lives, and they had come out safely thanks to their sensory and tactile apparatus, a partial loss of which is not fatal.

After these biological remarks we shall discuss the structure

of the integumental appendages of the larvae of H. caraboides.

The greater part of the hairs are chaetoids, i. e. organs developed exclusively from the chitinous cuticula ; the hypoderm under them does not show any peculiarities in its structure (Pl. 27, fig. 7).

The terminal (long) hairs of the pleural appendages are also chaetoids, but they take their origin from a differentiated terminal platform under which there are located large cells with large nuclei. These cells are probably of a neural character ; but this could have been proved only by application of special methods of staining (with methylene-blue for instance), which did not enter into the task of my work.

The setae of the integument are distinguished by greater length, stoutness, and stiffness (Pl. 27, fig. 8). The base of the seta is lodged in a chitinous cup-like tubercle (theca, *ct*) in the interior of which is found a differentiated ring, which is stained black by iron haematoxylin. Under this chitinous armature lie two cells (fig. 8, *ctc*), of which the larger one is trichogenous and the smaller thecogenous. Together they form a kind of follicle which invaginates into the cavity of the body from a row of hypodermal cells.

The setae described perfectly correspond with the type of dermatochaetae, according to the classification of the integumental appendages given by N. Nassonov (1901). In particular they belong to the dermatochaetae plerothecatae (E. Pavlovsky, 1917), i. e. setae with a solid theca.

Finally, the largest setae (Pl. 27, fig. 9, on a pigmented base) are distinguished by the most complicated structure. The long chitinous rod (fig. 9, tc_1) has its base set into a barrel-like elevation of the integument. Into the upper part of this barrel a short cylindrical cartridge (fig. 9, tc_2) is inserted, with which, properly speaking, the seta is articulated.

Under this formation there lie two large cells (*tc*), one of which is distinguished by an enormous nucleus (the trichogenous cell) ; in the protoplasm the borders between the nuclei are not visible.

The hypodermal cells (Pl. 27, fig. 9, *hp*) are distinguished from the ones just described by a smaller size. In the hollow of the cartridges there is a trace of lighter protoplasm directly connected with the protoplasm of the trichogenous cell. In this place there are located cells which stain pale and have nuclei containing but little chromatin, and which are evidently the nuclei of the hypoderma of the cartridges. From beneath this structure emerges a rather stout sinuated fibre, which terminates in a darker widening with a slender terminal appendage (Pl. 27, fig. 9, *n ?* a nervous fibre ?) : the nervous fibre reaches from the body-cavity to the trichogenous cell (Pl. 27, fig. 9, *ns ?*).

These setae can be characterized as dermatochaetae duplithecatae, as their basal cup (the theca) is double.

In conclusion, a few more words must be said regarding the structure of the pleural appendages of H. caraboides. The hypoderm and the chitinous cuticula are not distinguished by any particular peculiarities from the usual integument of the remaining parts of the body. They are covered with chaetoids : the terminal long hairs are connected at their bases with nervous cells.

The muscles are fastened to the lateral wall (Text-fig. 14, *m*) of the pleural appendage and their function is to contract the appendage. Through the lumen of the latter pass slender and few tracheae and the fat-body ; besides the indispensable cells of the haemolymph are present.

On the Nervous System.

The central nerve-cord consists, excluding the cephalic ganglia, of three pairs of thoracic and eight abdominal ganglia, of which the last ones, i. e. the tenth and eleventh, almost touch each other, and the ninth is situated nearer to the tenth than to the eighth. The thoracic ganglia are larger than the abdominal ones : the first abdominal ganglion closely touches the last pair of thoracic ganglia. On the whole the ganglia are disposed fairly equally, and the whole cord shows a more or less regular structure.

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DESCRIPTION OF PLATE 27.

Fig. 1.—Transverse section through rectal sac of mature larva of Hydrophilus caraboides at the anterior end; high (*ep*) and low (*epp*) epithelial walls are visible. In the cavity of the sac are bits of torn and ground chitin (*ch*), swallowed together with liquid parts of food. Duboscq, iron haematoxylin. Zeiss, ob. AA, oc. 2.

Fig. 2.—Same, but in a larva of the first stage. Duboscq, iron haematoxylin. Zeiss, ob. AA, oc. 2.

Fig. 3.—Part of longitudinal section of middle intestine (stomach) with crypts (*crp*) of a larva of first stage. *mt*, circular muscles; *ml*, longitudinal muscles. Duboscq, Giemsa's stain. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 1.

Fig. 4.—Crypt of stomach of mature larva. Duboscq, iron haematoxylin. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 1.

Fig. 5.—Oblique section of Malpighian tubule of mature larva. In the protoplasm of cells conspicuous differentiation of layers and copious accumulation of pigment grains (*pg*). Duboscq, Unna's Polychr.-Methylenblau. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 0.

Fig. 6.—Same, in a larva of first stage. Duboscq, iron haematoxylin. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 0.

Fig. 7.—Hair (*cht*) of chaetoid type from back of larva. Under the hair is the usual hypodermic cell (*hp*). *ch*, chitine. Duboscq, iron haematoxylin. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 2.

Fig. 8.—Hair with thaeca (*tc*) and ampulla (*ctc*) consisting of two—the thaecogenous and trichogenous—cells. *ch*, chitin; *hp*, hypodermic cell; *cht*, chaetoid. Duboscq, Giemsa's stain. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 2.

Fig. 9.—Hair (*tr*) with double thaeca (tc_1, tc_2) and attending nerve (*ns* ? *n* ?); *tc*, ampulla; *hp*, hypodermic cell; *cht*, chaetoid. Duboscq, iron haematoxylin. Zeiss, ob. $^1/_{12}$ hom. imm., oc. 4.